TP 295

# Triple Quadrupole Mass Spectrometer Performance: Evaluation and Mitigation of Charging

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## Abstract

**Purpose:** The charging of quadrupole systems upon exposure to proteomic samples via nano-liquid chromatography (nLC) can be a problem for quantitation and stability. Here, we assess quadrupole charging and explore potential mitigation strategies.

**Methods:** Mixtures of digested human plasma (1 to 5 µg) and Pierce™ Peptide Retention Time Calibration Mixture (PRTC, 0.1 pmol) were sampled with a Thermo Scientific™ Ultimate™ 3000 UHPLC flowing at 1 µL/min and introduced via a EP906 PepMap™ column onto a Thermo Scientific™ TSQ Altis™. Three representative compounds with a minimum of two SRM transitions of mid range mass were assessed to evaluate charging.

**Results:** We were able to forensically identify the sources of these effects and extend the overall performance lifetime. Additionally, we demonstrated the ability to increase proteomics sample loading with no appreciable signal degradation.

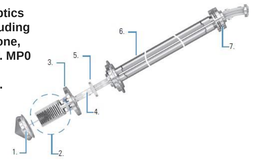
## Introduction

**TSQ** instruments are used to quantify components of samples with high specificity. When used for analysis of highly non-volatile compounds such as peptides, instruments can accumulate material on critical focusing elements which in-turn results in the accumulation of positive and negative charges on the devices. This changes the electric fields needed to transmit ions, resulting in a decrease in signals and generally poor system performance. Here we explore devices that most strongly influence signal degradation and test methodologies to extend system lifetime and performance.

We carried out a two-phase study. Phase 1 was exploratory with device voltages set just past onset for transmission. This allows us to more quickly identify device failures, and later, in Phase 2, set to potentials much further past onset for transmission. Our metric was primarily the amount loaded that resulted in a 50% loss in signal.

Some key devices are indicated in Figure 1, with a focus on lens L0, MP0 ion optics, and the analytical quadrupole including pre- and post- filtering segments.

**Figure 1. The Altis source and front-end optics of the quadrupole mass spectrometer including the Q1 quadrupole are shown. 1: Sweep cone, 2: RF lens, 3. MP00 multiple and lens L0, 4. MP0 Ion Optics, 5. Neutral blocker, 6. Quadrupole with pre- and post-filters , 7. Entrance to Q2.**




## Materials

### Sample Preparation

Stock PRTC (1pmol/uL): 160 µL of 0.1% formic acid in water was transferred to a 300 uL polypropylene vial. 40 µL of 5 pmol/µL Pierce PRTC stock solution was added to each vial. The vial was vortexed and stored in a -30C freezer until it was ready to be analyzed.

Sample: 100µg of trypsin digest plasma (Pierce) were removed from a -30°C freezer and allowed to warm to room temperature. Four mixes (ID: 1-4) for analysis were prepared according to the table below.

**Table 1. Mix identification (Mix ID) for PRTC and plasma samples used in this work**

| Mix ID | 0.1% FA in H2O (µL) | PRTC stock (µL) | PRTC in vial pmol/µL | Plasma in vial (µg/µL) | Injection volume (µL) | Plasma on column (µg) |
|---|---|---|---|---|---|---|
| **1** | 190 | 10 | 0.05 | 0.5 | 2 | **1** |
| **2** | 152 | 8 | 0.05 | 0.625 | 2 | **1.25** |
| **3** | 90 | 10 | 0.1 | 1.0 | 2 | **2** |
| **4** | 96 | 4 | 0.04 | 1.0 | 5 | **5** |

## Methods

### Test Methods

Quantitative analysis of Mixes 1-4 (Table 1) was carried out using a Thermo Scientific Ultimate 3000 UHPLC (Catalog number: ULTIM3000RSLCNANO) flowing at 1 µL/min with a 1-98% gradient (A: 0.1% Formic Acid in UHPLC grade water, B: 80% acetonitrile in UHPLC grade water). The trap column was intentionally omitted to maximize peptide deposition.

The run-time for each sample was 69 minutes. MS data was collected on a Thermo Scientific TSQ Altis Triple Quadrupole Mass Spectrometer (Catalog number: TSQ03-10002) with a ES906 Thermo Scientific PepMap 100Å C18 Column held at 45°C. Selected reaction monitoring (SRM) was used for 15 peptides in PRTC and over 100 peptides in human plasma. Reported below are the three compounds used to monitor system performance. Collision energies were set to the same value for each transition within peptides.

**Table 2. SRM transitions for three selected compounds in positive ion mode**

| Peptide | Source | Precursor | Transitions | CE |
|---|---|---|---|---|
| **TASEFDSAIAQDK (2+)** | PRTC | 695.832 | 740.403, 855.43, 1002.50, 1218.57 | 25.8 |
| **TATSEYQTFFNPR (2+)** | Plasma | 781.367 | 272.172, 386.215 | 28.8 |
| **TCPKPDDLPFSTVVPLK** | Plasma | 957.505 | 357.250, 987. 587 | 34.8 |

### Data Collection, Processing and Interpretation

Thermo Scientific Xcalibur™ data analysis software was used for data acquisition. Skyline[2] was used for data processing and files were uploaded using AutoQC into PanoramaWeb.

Weekly diagnostics were collected on the MS system using Pierce's Extended Mass Range Solution (EMRS) to evaluate Q1/Q3 performance. These included monitoring of the EMRS components in Full Scan mode, charging tests, and individual device diagnostics.

**Figure 2. Example chromatograms and retention times for all PRTC components and the two plasma peptides (red inset) chosen as representative as a component of the overall total ion chromatogram. The PRTC compound selected for quantitation is identified with a red asterisk.**

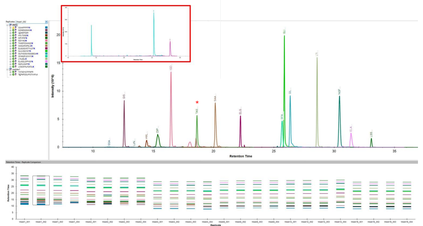
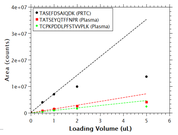
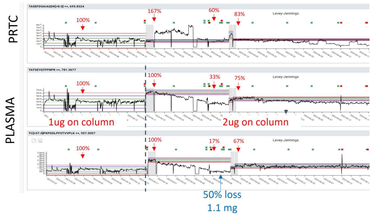


**Figure 3: At right, the calibration curves for PRTC and two selected human plasma peptides were collected by varying the amount injected for Mix 4 (See Table 1)**
Here, deviation from linearity for PRTC occurs at 0.04 pmol (1 µL) and 2 µg (2 µL) for plasma). Thus, we were operating in slight saturation for PRTC (40%) and in the linear regime for plasma.




## Results: Phase 1 - Initial accumulation

**Figure 4. Summary of Phase 1 exposure campaign. The noted percentages are the remaining signal (weighted for injection amount). Here, samples were prepared with 1 µg and 2 µg on column. 50% loss in PRTC was seen after 1.1 mg (750 injections) of plasma on column.**




Following the initial experiments of Figure 4, we pushed the system to full failure (<10% initial peptide amplitude) by loading 5 µg in each injection with Mix ID 4. After 2.7 mg of total plasma (900 total injections), we compared charging and device transmission curves between the contaminated system and the clean (post-contamination) system. In this instance, accumulation were visible on the quadrupole with dimensions of 3 x 2 mm (not shown).

**Figure 5. Charging Tests show changes in ion transmission when devices charge. In a clean system (left) devices all transmit with similar amplitudes. For our heavily contaminated system after 2.7 mg, Q1 transmission was 10x weaker than Q3, indicating accumulation on Q1 devices.**

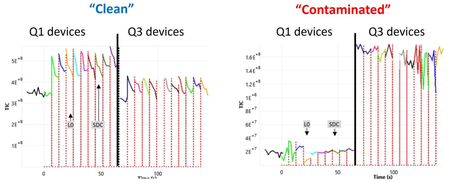
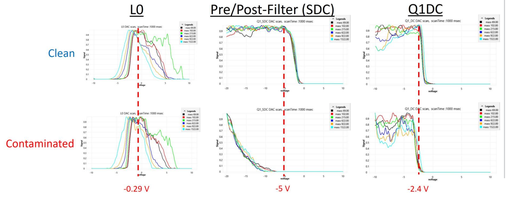


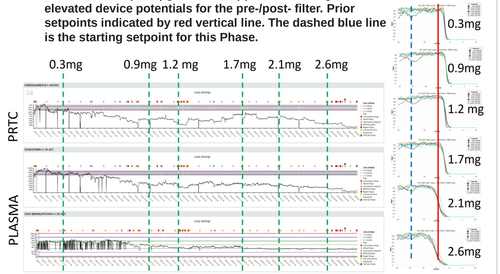

**Figure 6. Transmission of ions as a function of device voltage (red dashes) shown for L0 (L0 in Q1 devices), Pre/Post filter (SDC in Q1 devices) and Q1 DC. All devices were compared, but only the pre/post-filter would require voltage adjustment of more than -5V. The Q1 DC would require adjustment of -0.2V. L0 would not require any changes.**




## Results: Phase 2 - Minimize Charging

Following a full system clean of the heavily contaminated system, we resumed the deposition experiment with a moderate loading (Mix ID 2: 1.25 µg plasma on column, linear response) and now lowered the pre-/post-filter potentials to -15V (blue dashed line in Figure 7. All other device voltages were set the same.

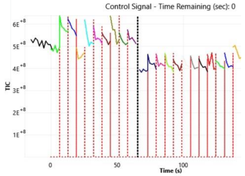
**Figure 7. The exposure result during the phase 2 campaign with moderate (1.25 µg on column) plasma loading and elevated device potentials for the pre-/post- filter. Prior setpoints indicated by red vertical line. The dashed blue line is the starting setpoint for this Phase.**




We noted some early losses after 1.5 mg, but these appeared to be more related to the LC. The diagnostics (see pre-/post-filter data at right of Figure 7, blue line) maintained a steady transmission. A column change after 1.2 mg improved the signal slightly, and after a two-week shutdown and restart, the signal quickly recovered on its own with no apparent changes in the charging or performance diagnostics. Thus, no cleaning steps were taken. The next measured 50% decrease in signal was measured around 2.1 mg and continued to drop from there. It should be noted that the declines were gradual, resulting in a still usable instrument.

The charging diagnostics (Figure 8) around the 2.1 mg total injected indicate that the downstream components are not influencing ion transmission in the same way we saw before. The small decrease in L0 was an early artifact of the system status and had no impact on performance.

**Figure 8. Charging test after 2.1 mg deposition with elevated pre-/post-filter voltages indicates no major charging issues as seen before.**




## Synopsis: Charging and accumulation

The accumulation of non-volatile ions (here, peptides) on key devices within the triple quadrupole family is a known consequence of nano-flow experiments. The result of the accumulation is found in charging within the instrument, which modifies the potentials experienced by the ions, thus changing their flight path to either collide on devices (enhancing accumulation) or to be lost elsewhere before reaching the detector.

The larger potentials applied on key analytical devices likely extends the up-time by spreading the accumulation further downstream. As we continue to contaminate our system, we intend to investigate any visual contamination compared to what we saw before.

## Results: Summary

**Results:** At the outset of the campaign, our instrument was configured for transmission of MS devices to accelerate performance losses over time. With these initial settings, we observed a 50% drop in signal after 1.1 mg total on column (750 injections varied between 1 µg to 2 µg loading). Diagnostics collected on all devices were used to identify potentially contaminated components. Stepwise cleaning included replacement of the LC column, cleaning or replacing the ion transfer tube, cleaning the source optics, cleaning through the ion transfer guide, and cleaning the first analytical quadrupole. This latter step returned the system to improved operation.

Following the initial decrease, we wanted to examine a heavily contaminated system, so we re-initiated with moderate recovery of performance and proceeded until we achieved 10% of the initial signal. This was around 2.7 mg total on column (900 injections at 2 µg to 5 µg loading). Inspection showed us heavy contamination on the analytical quadrupole.

We then fully cleaned and re-started with the higher potentials to evaluate if we could extend the operational lifetime. Under these conditions, our performance has been extended to more than 2 mg in total introduced with limited signal degradation. This is likely due to reducing the density of unwanted material accumulation on key optical elements.

We anticipate using the device diagnostic curves as part of an instrumental monitoring procedure that will provide predictive feedback for needed preventative maintenance. Application of such an approach will improve the up-time for customers as service visits can be targeted to specific device components that are known to become contaminated, leading to faster recovery of system performance.

## Conclusions

- When plasma and other peptide heavy samples are sampled with a LC/MS system, it is imperative to control accumulation of these materials inside the spectrometer to minimize downtime. This can be done by adjusting device settings to settings beyond threshold where ion transmission is constant. SIMION models might be used to simulate the effect of contamination and guide choices for device settings.
- Specific device diagnostics can be used in future efforts to predict pending contamination and be used to warn the operator of needed preventative maintenance.
- Matrix effects can be minimized with appropriate trap columns and device settings constructed to improve repeatability and system up-time.

## References

1. **TSQ Altis, TSQ Quantis, and TSQ Fortis Hardware Manual**. 80111-98005 Rev. A (2019)
2. Pino, L.K., Searle, B.C., Bollinger, J.G., Nunn, B., MacLean, B., MacCoss, M.J. The Skyline ecosystem: Informatics for quantitative mass spectrometry proteomics. *Mass Spectrom Rev.*, 2020, May; **39**(3):229-244

## Acknowledgements

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